Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.8

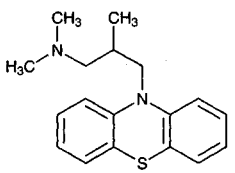
OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Trimeprazine



Molecular formula: $C_{18}H_{22}N_2S$
Molecular weight: 298.45
CAS Registry No.: 84-96-8, 4330-99-8 (tartrate)
Merck Index: 9834
Lednicer No.: 1 378

SAMPLE
Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Homogenize tissue with 12 volumes water. 1 mL Whole blood, urine, bile, gastric contents, or tissue homogenate + 5 mL n-heptane:isoamyl alcohol 98.5:1.5 + 500 μL pH 8.5 saturated sodium carbonate buffer + 20 μL 100 μg/mL prochlorperazine in MeOH, agitate, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL MeCN, inject a 70 μL aliquot.

HPLC VARIABLES
Column: 250 × 3.2 5 μm Spherisorb CN
Mobile phase: MeCN:15 mM pH 6.5 sodium acetate buffer 95:5
Flow rate: 1
Injection volume: 70
Detector: UV 254

CHROMATOGRAM
Internal standard: prochlorperazine
Limit of detection: 20 ng/mL

KEY WORDS
whole blood; brain; liver; kidney; heart; muscle

REFERENCE
Kintz,P.; Berthault,F.; Tracqui,A.; Mangin,P. A fatal case of alimemazine poisoning, *J.Anal.Toxicol.*, **1995**, *19*, 591–594.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 10 μm Micropak CN (Varian)
Mobile phase: MeCN:20 mM ammonium acetate 90:10
Flow rate: 2.5
Injection volume: 50
Detector: UV 254 or E, Bioanalytical Systems LC4A, glassy carbon electrode +0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.6
Limit of detection: 0.1 ng/mL (electrochemical)

OTHER SUBSTANCES
Extracted: amitriptyline, butaperazine, chlorpromazine, fluphenazine, promazine, promethazine, thioridazine, trifluoperazine
Simultaneous: acetophenazine, benztropine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, thiothixene, trihexyphenidyl
Interfering: carphenazine, triflupromazine

KEY WORDS
plasma; whole blood

REFERENCE
Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1-5 mL Plasma + 1 mL 1 M NaOH, extract with mixed hexanes for 30 min, centrifuge. Remove a 9 mL aliquot of the hexane layer and evaporate it to dryness under a stream of nitrogen at 30°, dissolve residue in 100 μL mobile phase, inject a 50 μL aliquot. Whole blood. 10 mL Whole blood + 1 mL 1 M NaOH, extract with 15 mL mixed hexanes for 1 h. Remove an aliquot of the hexane layer and evaporate it to dryness, reconstitute the residue in 1 mL 100 mM HCl, extract with 5 mL chloroform by vortexing for 1 min, centrifuge. Remove a 4.5 mL aliquot of the chloroform layer, evaporate to dryness, dissolve in 10 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 10 μm Micropak CN
Mobile phase: MeCN:5 mM ammonium acetate 90:10 (vary ammonium acetate concentration to achieve best separation)
Flow rate: 2.5
Injection volume: 10-50
Detector: UV 254 or E, Bioanalytical Systems LC-4A, glassy carbon electrode +0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 17.0
Limit of detection: 0.1 ng/mL (E), 10 ng/mL (UV)

OTHER SUBSTANCES
Extracted: acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, piperacetazine, orphenadrine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl

KEY WORDS
plasma; whole blood

REFERENCE
Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 1 mL 100 ng/mL prochlorperazine in water + 500 μL saturated sodium carbonate solution, vortex for 5 s, add 5 mL pentane:isopropanol 97:3, shake for 15 min, centrifuge at 1725 g for 10 min, repeat the extraction. Combine the organic layers and evaporate them to dryness at 65°, reconstitute the residue in 200 μL MeCN, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax CN
Mobile phase: MeCN:100 mM ammonium acetate buffer 90:10
Flow rate: 4
Injection volume: 100
Detector: E, Bioanalytical Systems, +0.9 V

CHROMATOGRAM
Retention time: 2.74
Internal standard: prochlorperazine (5.56)
Limit of detection: 0.125 ng/mL
Limit of quantitation: 0.25 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
McKay,G.; Cooper,J.K.; Midha,K.K.; Hall,K.; Hawes,E.M. Simple and sensitive high-performance liquid chromatographic procedure with electrochemical detection for the determination of plasma concentrations of trimeprazine following single oral doses, *J.Chromatogr.*, **1982**, *233*, 417–422.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Whole blood + 1 mL 1 M NaOH + 10 mL hexanes, extract for 30 min, repeat extraction. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, shake gently for 10 min or vortex for 1 min, centrifuge. Remove 4.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 10 μL mobile phase, inject an aliquot (J. Chromatogr. 1982, 231, 361).

HPLC VARIABLES
Column: 10 μm Micropak CN
Mobile phase: MeCN:100 mM ammonium acetate 90:10
Flow rate: 2
Detector: E, Bioanalytical Systems LC-4A, glassy carbon electrode +0.9 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 4.4
Internal standard: imipramine (6.8)
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, butaperazine, chlorpromazine, fluphenazine, promazine, thioridazine, trifluoperazine
Simultaneous: acetophenazine, benztropine, carphenazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, thiothixene
Interfering: promethazine, triflupromazine, trihexyphenidyl

KEY WORDS
whole blood

REFERENCE
Hu,O.Y.; Gfeller,E.; Perrin,J.H.; Curry,S.H. Relative bioavailability of trimeprazine tablets investigated in man using HPLC with electrochemical detection, *J.Pharm.Pharmacol.*, **1986**, *38*, 172–176.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood + 5 mL chloroform:isopropanol:n-heptane 60:14:26 + 1.5 mL saturated ammonium chloride solution (pH 9.5), agitate horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPak C18
Mobile phase: MeOH:THF:10 mM pH 2.6 KH_2PO_4 65:5:30
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 5.87
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
whole blood

REFERENCE
Kintz,P.; Berthault,F.; Tracqui,A.; Mangin,P. A fatal case of alimemazine poisoning, *J.Anal.Toxicol.*, **1995**, *19*, 591–594.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 255

CHROMATOGRAM
Retention time: 8.35
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil;

lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF

Sample preparation: 200 μL Serum, plasma, or CSF + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, elute the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine hydrochloride and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 30 × 2.1 40 μm preparative grade C18 (Analytichem); B 250 × 4.6 10 μm Partisil C8

Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 15 min, maintain at 30:70 for 4 min.

Column temperature: 50

Flow rate: 1.5

Detector: UV 280 for 5 min then UV 254

CHROMATOGRAM

Retention time: 7.53

Internal standard: heptanophenone (19.2)

OTHER SUBSTANCES

Extracted: acetazolamide, ampicillin, bromazepam, caffeine, carbamazepine, chloramphenicol, chlorothiazide, diazepam, droperidol, ethionamide, furosemide, isoniazid, methadone, penicillin G, phenobarbital, phenytoin, prazepam, propoxyphene, pyrazinamide, rifampin, trimethoprim

KEY WORDS

plasma; serum; column-switching

REFERENCE

Seifart,H.I.; Kruger,P.B.; Parkin,D.P.; van Jaarsveld,P.P.; Donald,P.R. Therapeutic monitoring of antituberculosis drugs by direct in-line extraction on a high-performance liquid chromatography system, *J.Chromatogr.*, **1993**, *619*, 285–290.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 253.5

CHROMATOGRAM
Retention time: 15.257

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject 75-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco
Mobile phase: EtOH:MeCN:t-butylamine 98:2:0.05 (Prepared from 1 gal EtOH + 77 mL MeCN + 1.9 mL t-butylamine.)
Flow rate: 2
Injection volume: 75-100
Detector: UV 254

CHROMATOGRAM
Retention time: 2.6
Internal standard: promazine (5.2)

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, amitriptyline, amoxapine, amphetamine, buprion, chlordiazepoxide, chlorimipramine, chlorpheniramine, chlorpromazine, cocaine, codeine, demoxepam, desipramine, desmethylchlordiazepoxide, desmethyldisopyramide, desmethyldoxepin, dextropropoxyphene, diazepam, disopyramide, doxepin, fluphenazine, hydroxyamoxapine (7- and 8-), 2-hydroxydesipramine, 2-hydroxyimipramine, 10-hydroxynortriptyline, iminostilbene, imipramine, iprindole, maprotiline, meperidine, methadone, morphine, nortriptyline, norzimeldine, oxapam, oxaprotiline, perphenazine, phentermine, procainamide, prochlorperazine, prolixin, promethazine, propoxyphene, protriptyline, pyrilamine, quinidine, thioridazine, trifluoperazine, triflupromazine, trimipramine, zimeldine
Noninterfering: thiopropazine
Interfering: loxepin, mianserin

KEY WORDS
normal phase

REFERENCE
Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279–292.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.07

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 5.8

OTHER SUBSTANCES
Simultaneous: amitriptyline, atropine, butalbital, chlorpromazine, desipramine, desmethylmaprotiline, doxepin, imipramine, maprotiline, methadone, norpropoxyphene, nortriptyline, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, protriptyline, trimipramine
Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil
Interfering: N-propionylprocainamide, quinidine, trifluoperazine

REFERENCE
Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 50 μL aliquot of a 20 mg/mL solution. Collect eluted enantiomers by switching column effluent onto one of two 100 × 4.6 25-40 μm Lichroprep RP18 columns. Wash these columns with 2.5 mL water, wash with 5 mL MeCN:water 10:90, elute with MeCN: 0.14% trifluoroacetic acid in water 70:30, evaporate eluate to give the enantiomers as their trifluoroacetate salts.)

HPLC VARIABLES
Column: 100 × 4 5 μm SGE-100GLC4-C8-30/5 octylsilica (Scientific Glass Engineering)
Mobile phase: MeCN:buffer 10:90 containing 9 g/L β-cyclodextrin (Buffer was 0.8% triethylamine adjusted to pH 4 with glacial acetic acid.)
Injection volume: 50
Detector: UV

CHROMATOGRAM
Retention time: 8.5, 10 (enantiomers)

KEY WORDS
chiral; preparative

REFERENCE

Cooper,A.D.; Jefferies,T.M. On-line recovery of trimeprazine enantiomers following chiral separation by reversed-phase high-performance liquid chromatography using a β-cyclodextrin-containing mobile phase, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 847–851.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 14.91 (A), 7.07 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE

Matrix: solutions
Sample preparation: Make up a 500 ng/mL solution in mobile phase, inject an aliquot.

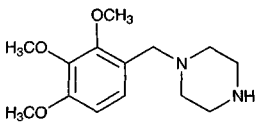
HPLC VARIABLES
Column: 250 × 4.6 5 μm Sumchiral OA-4700 (S)-tert-leucine-(R)-1-(α-naphthyl)ethylamine (YMC)
Mobile phase: Hexane:1,2-dichloroethane:EtOH:trifluoroacetic acid 800:150:100:1
Flow rate: 1
Injection volume: 100
Detector: F ex 254 em 280 (filter)

CHROMATOGRAM
Retention time: 5.73, 6.26 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ponder,G.W.; Butram,S.L.; Adams,A.G.; Ramanathan,C.S.; Stewart,J.T. Resolution of promethazine, ethopropazine, trimeprazine and trimipramine enantiomers on selected chiral stationary phases using high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *692*, 173–182.

Trimetazidine



Molecular formula: $C_{14}H_{22}N_2O_3$
Molecular weight: 266.34
CAS Registry No.: 5011-34-7, 13171-25-0 (2.HCl)
Merck Index: 9835

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

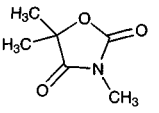
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 6.06

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Trimethadione



Molecular formula: $C_6H_9NO_3$
Molecular weight: 143.14
CAS Registry No.: 127-48-0
Merck Index: 9836
Lednicer No.: 1 232

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 50 μL α-methyl-α-propylsuccinimide in MeOH, shake for 2 min, centrifuge at 1500 g for 5 min, inject a 10-20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Shodex ODSpak F-411A
Mobile phase: MeCN:water:PIC-B5 (low UV) 15:85:3.5
Injection volume: 10-20
Detector: UV 200

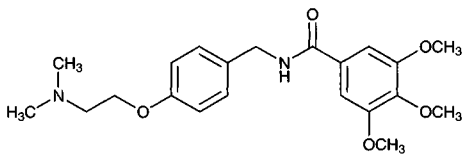
CHROMATOGRAM
Retention time: 5.5
Internal standard: α-methyl-α-propylsuccinimide (12.7)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: acetazolamide, carbamazepine, pentobarbital, phenobarbital, phenytoin, primidone

KEY WORDS
serum; rat; human; pharmacokinetics

REFERENCE
Tanaka,E.; Hagino,S.; Yoshida,T.; Kuroiwa,Y. Simultaneous determination of trimethadione and its metabolite in rat and human serum by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *308*, 393–397.

Trimethobenzamide



Molecular formula: $C_{21}H_{28}N_2O_5$
Molecular weight: 388.46
CAS Registry No.: 138-56-7, 554-92-7 (HCl)
Merck Index: 9839
Lednicer No.: 1 110

SAMPLE
Matrix: blood
Sample preparation: 2 mL Serum or plasma + 100 μL 10 μg/mL trimethobenzamide in MeOH + 1 mL buffer + 10 mL n-butyl chloride:isopropanol 95:5, shake for 10 min, centrifuge at 500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 200 μL chloroform and 200 μL 25 mM HCl, vortex for 10 s, centrifuge at 500 g for 3-5 min, inject a 50-60 μL aliquot of the upper aqueous layer. (Buffer was 630 mL of a solution containing 1 M boric acid and 1 M KCl + 370 mL 1 M sodium carbonate, adjust pH to 9.0.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyanopropyldimethylsilyl (PCN) (Supelco)
Mobile phase: MeCN:0.06% phosphoric acid 15:85 containing 0.01% octylamine (After analysis wash out system with MeCN:water 20:80.)
Flow rate: 2
Injection volume: 50-60
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: trimethobenzamide

OTHER SUBSTANCES
Extracted: encainide
Simultaneous: dipyridamole, oxazepam
Noninterfering: amiodarone, caffeine, chloral hydrate, chlordiazepoxide, diazepam, ethosuximide, flecainide, lidocaine, methadone, mexiletine, nicotine, phenobarbital, phenytoin, primidone, procainamide, propranolol, quinidine, tocainide, tricyclic antidepressants

KEY WORDS
plasma; serum; trimethobenzamide is IS

REFERENCE
Dasgupta,A.; Rosenzweig,I.B.; Turgeon,J.; Raisys,V.A. Encainide and metabolites analysis in serum or plasma using a reversed-phase high-performance liquid chromatographic technique, *J.Chromatogr.*, **1990**, *526*, 260–265.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.9

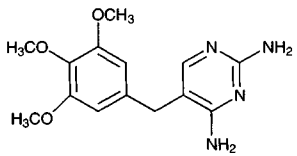
OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine,

methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Trimethoprim



Molecular formula: $C_{14}H_{18}N_4O_3$
Molecular weight: 290.32
CAS Registry No.: 738-70-5
Merck Index: 9840
Lednicer No.: 1 262

SAMPLE

Matrix: blood
Sample preparation: Add 1.5 mL MeCN to 500 µL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 µL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 µm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 µm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)
Mobile phase: MeOH:20 mM pH 4 phosphate buffer 38:62
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 245, F ex 270 em 389

CHROMATOGRAM

Retention time: 1.9

OTHER SUBSTANCES

Extracted: triamterene

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 75 μL acetone:10% trichloroacetic acid 1:2, vortex for 5 s, centrifuge for 4 min. Remove 62.5 μL of the supernatant and add it to 62.5 μL 50 mM KH_2PO_4, add 250 μL diethyl ether, vortex for 10 s, centrifuge for 5 min, filter (0.45 μm) the lower aqueous layer, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 75 × 4.6 TSK gel ODS-80TM (Tosoh)
Mobile phase: MeCN:50 mM pH 6.0 KH_2PO_4 8:92
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 30
Internal standard: trimethoprim

OTHER SUBSTANCES
Extracted: vancomycin

KEY WORDS
serum; trimethoprim is IS

REFERENCE
Morishige,H.; Shuto,H.; Ieiri,I.; Otsubo,K.; Oishi,R. Instability of standard calibrators may be involved in overestimating vancomycin concentrations determined by fluorescence polarization immunoassay, *Ther.Drug Monit.*, **1996**, *18*, 80–85.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 8.282

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 31

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

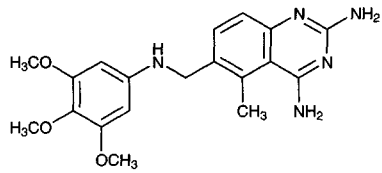
CHROMATOGRAM
Retention time: 27.5

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfabenzamide, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

Trimetrexate



Molecular formula: $C_{19}H_{23}N_5O_3$
Molecular weight: 369.42
CAS Registry No.: 52128-35-5, 82952-64-5 (glucuronate)
Merck Index: 9851
Lednicer No.: 4 149

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C2 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of water, do not allow to go dry. 1 mL Plasma + 160 ng IS, vortex, add to the SPE cartridge at 0.5 mL/min, wash with two 1 mL portions of water, dry under vacuum for 1 min, wash with 250 μL MeCN, dry under vacuum for 20 s, elute with 500 μL MeOH:water 95:5. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 400 μL mobile phase, vortex for 30 s, centrifuge at 1800 g for 15 min, inject a 100 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 7 μm Zorbax TMS
Column: 250 × 4.6 5 μm Zorbax TMS
Mobile phase: MeCN:buffer 23:77 (Buffer was 50 mM $(NH_4)H_2PO_4$ containing 0.8% triethylamine and 0.2% phosphoric acid, pH 4.5.)
Column temperature: 45
Flow rate: 1.2
Injection volume: 100
Detector: UV 241

CHROMATOGRAM

Retention time: 9.4
Internal standard: N6-phenylmethyl-2,4,6-quinazolinetriamine (12.0)
Limit of quantitation: 2.4 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites, cisplatin, cytarabine, doxorubicin, etoposide, 5-fluorouracil, folinic acid (leucovorin), lomustine, methotrexate, morphine, prednisolone, ribavirin, sulfamethoxazole, 6-thioguanine, trimethoprim, vinblastine, vincristine, zidovudine
Interfering: chlorambucil, melphalan

KEY WORDS

SPE; plasma; pharmacokinetics

REFERENCE

Bullen,W.W.; Chang,T.; Whitfield,L.R. High-performance liquid chromatographic assay for trimetrexate in human plasma, *J.Chromatogr.*, **1990**, *526*, 266–272.

SAMPLE

Matrix: blood, feces, urine
Sample preparation: Plasma. Condition a SPICE ODS SPE cartridge (Analtech) with 7.5 mL MeOH and 7.5 mL buffer. 1 mL Plasma + 1 mL buffer, add to SPE cartridge, wash with two 2.5 mL portions of buffer, dry under vacuum, elute with two 2.5 mL portions of MeOH:triethylamine 99:1. Evaporate the eluate to dryness under reduced pressure, reconstitute with 1 mL mobile phase, inject a 200 μL aliquot. Feces. Condition a SPICE ODS SPE cartridge (Analtech) with 7.5 mL MeOH and 7.5 mL water. Add dithiothreitol to feces (final concentration 1 mM), add an equal volume of water, add to SPE cartridge, wash with two 2.5 mL portions of MeOH:water 12.5:87.5, dry under vacuum, elute with two 2.5 mL portions of MeOH:triethylamine 99:1. Evaporate the eluate to dryness under reduced pressure, reconstitute with 1 mL mobile phase, add dithiothreitol (final concentration 1 mM), inject a 200 μL aliquot. Urine. Dilute 1:10 to 1:100 with 15 μM IS in mobile phase, inject an aliquot. (Buffer was 1.5% acetic acid adjusted to pH 5.5 with ammonium hydroxide.)

HPLC VARIABLES
Guard column: 5 μm ODS (Brownlee)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:50 mM NaH_2PO_4:acetic acid 17:82.2:0.8 adjusted to pH 5.5 with triethylamine
Column temperature: 20
Flow rate: 1
Injection volume: 200
Detector: E, Bioanalytical Systems LC-4A, 0.6 V or UV 254

CHROMATOGRAM
Retention time: 40
Internal standard: N,N-diethyl-5,7-dimethoxytryptamine hydrogen oxalate (35)
Limit of quantitation: 20 nM (feces), 50 nM (plasma, urine)

KEY WORDS
plasma; SPE; pharmacokinetics

REFERENCE
Lin,J.T.; Cashmore,A.R.; Baker,M.; Dreyer,R.N.; Ernstoff,M.; Marsh,J.C.; Bertino,J.R.; Whitfield,L.R.; Delap,R.; Grillo-Lopez,A. Phase I studies with trimetrexate: clinical pharmacology, analytical methodology, and pharmacokinetics, *Cancer Res.*, **1987**, *47*, 609–616.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 500 mg C18 Bond Elut SPE cartridge with three column volumes of MeOH and three column volumes of water. Plasma, serum. Centrifuge plasma for 3-5 min. Add 1 mL serum or plasma to the SPE cartridge, wash with 6 mL water, wash with 1.5 mL MeCN, wash with 500 μL water, elute with 1 mL MeOH:80 mM sodium citrate 95:5. Filter (0.45 μm) the eluate, inject a 10 μL aliquot of the filtrate. Urine. Add 1 mL urine to the SPE cartridge, wash with 6 mL water, wash with 1 mL MeCN, wash with 1 mL MeOH:20 mM pH 4.5 sodium acetate 25:75, elute with 1.25 mL MeOH:80 mM sodium citrate 95:5. Filter (0.45 μm) the eluate, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 30 × 4.6 Spherisorb RP-18
Column: 300 × 3.9 10 μ μBondapak C18
Mobile phase: MeCN:Buffer 40:60 (Buffer was water containing 0.02% phosphoric acid and 0.08% triethylamine.)
Flow rate: 2
Injection volume: 10
Detector: UV 241

CHROMATOGRAM
Retention time: 4.5
Limit of quantitation: 50 ng/mL (urine), 20 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; serum; SPE; human; mouse

REFERENCE
Ackerly,C.C.; Hartshorn,J.; Tong,W.P.; McCormack,J.J. A rapid and sensitive method for determination of trimetrexate from biological fluids, *J.Liq.Chromatogr.*, **1985**, *8*, 125–134.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 500 mg C18 Bond Elut SPE cartridge with three column volumes of MeOH and three column volumes of water. Plasma, serum. Centrifuge plasma for

3-5 min. Add 1 mL serum or plasma to the SPE cartridge, wash with 6 mL water, wash with 1.5 mL MeCN, wash with 500 μL water, elute with 1 mL MeOH:80 mM sodium citrate 95:5. Filter (0.45 μm) the eluate, inject a 10 μL aliquot of the filtrate. Urine. Add 1 mL urine to the SPE cartridge, wash with 6 mL water, wash with 1 mL MeCN, wash with 1 mL MeOH:20 mM pH 4.5 sodium acetate 25:75, elute with 1.25 mL MeOH:80 mM sodium citrate 95:5. Filter (0.45 μm) the eluate, inject a 10 μL aliquot of the filtrate (J.Liq.Chromatogr. 1985, 8, 125).

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Aquapore C18
Column: 100 × 4.6 5 μm Spherisorb ODS
Mobile phase: Gradient. MeCN:buffer from 15:85 to 40:60 over 10 min, re-equilibrate at initial conditions for 10 min. (Buffer was 0.08% triethylamine containing 0.04% phosphoric acid.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 241

CHROMATOGRAM
Retention time: 7.6
Limit of quantitation: 100 ng/mL

KEY WORDS
plasma; serum; SPE

REFERENCE
Hudes,G.R.; LaCreta,F.; DeLap,R.J.; Grillo-Lopez,A.J.; Catalano,R.; Comis,R.L. Phase I clinical and pharmacologic trial of trimetrexate in combination with 5-fluorouracil, *Cancer Chemother.Pharmacol.*, **1989**, *24*, 117–122.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injection 1:5 with MeOH:water 50:50, inject a 2 μL aliquot.

HPLC VARIABLES
Column: 100 × 2 5 μm MOS C8 (Hewlett-Packard)
Mobile phase: MeCN:MeOH:50 mM $(NH_4)H_2PO_4$ 18:12:70
Flow rate: 0.5
Injection volume: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 2.03

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; water

REFERENCE
Stetson,P.L.; Shukla,U.A.; Ensminger,W.D. Stability of trimetrexate, a new non-classical antifolate, in infusion solutions, *J.Chromatogr.*, **1989**, *464*, 163–171.

SAMPLE
Matrix: urine
Sample preparation: Condition a 1 mL 100 mg Bond Elut CN SPE cartridge with 3 mL MeCN and 3 mL water. 1 mL Urine + 100 μL 20 μg/mL trimethoprim in water + 1 mL water, add to the SPE cartridge, wash with 3 mL water, elute with 1 mL MeCN:water 15:85 containing 0.75% triethylamine and 0.375% phosphoric acid (85%), inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Aquapore C18 (Brownlee)

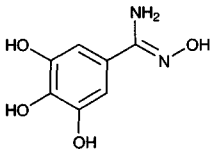
Column: 100 × 4.6 5 μm Hypersil ODS C18
Mobile phase: Gradient. MeCN:buffer from 9:91 to 35:65, re-equilibrate at initial conditions for 5 min. Buffer was 0.16% triethylamine containing 0.08% orthophosphoric acid (85%), pH 4.2.)
Flow rate: 1.5
Injection volume: 200
Detector: UV 241

CHROMATOGRAM
Retention time: 11.0
Internal standard: trimethoprim (5.8)
Limit of quantitation: 100 ng/mL

KEY WORDS
SPE

REFERENCE
Tinsley,P.W.; LaCreta,F.P. Improved chromatographic method for the determination of trimetrexate in urine, *J.Chromatogr.*, **1990**, *529*, 468–472.

Trimidox



Molecular formula: $C_7H_8N_2O_4$
Molecular weight: 184.15

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 0.1 mM solution in 10 mM KH_2PO_4, adjust pH to 6 with a few drops 5 M KOH or phosphoric acid, inject a 20 μL aliquot.

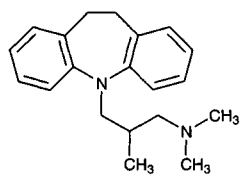
HPLC VARIABLES
Column: 150 × 4.6 3 μm Supelcosil LC18
Mobile phase: MeOH:buffer 5:95 (Buffer was 0.05% triethylamine adjusted to pH 6 with 50 mM phosphoric acid.)
Flow rate: 0.5
Injection volume: 20
Detector: UV 255

OTHER SUBSTANCES
Simultaneous: amidox, didox

KEY WORDS
comparison with DC polarography and UV spectrophotometry

REFERENCE
Romanova,D.; Vachalkova,A.; Szekeres,T.; Elford,H.L.; Novotny,L. The new inhibitors of ribonucleotide reductase -comparison of some physico-chemical properties, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 951–956.

Trimipramine



Molecular formula: $C_{20}H_{26}N_2$
Molecular weight: 294.44
CAS Registry No.: 739-71-9, 521-78-8 (maleate)
Merck Index: 9852

SAMPLE
Matrix: blood
Sample preparation: Condition a 15 mg 3 mL PLUS.MPI (Ansys, USA) SPE disc with 200 μL MeOH and 200 μL 100 mM pH 6.0 potassium phosphate monobasic, do not allow to dry. Mix 1 mL serum with 30 μL 10 μg/mL IS in water, add 1 mL 100 mM pH 6.0 potassium phosphate monobasic buffer, mix well. Add the sample to the SPE disc, wash with 500 μL 1 M acetic acid, wash with 500 μL MeOH, dry under vacuum for 5 min. Elute with two 300 μL portions of MeCN:triethylamine 100:2. Evaporate the eluate under a stream of nitrogen, dissolve the residue in 800 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 1 opti-guard column RP C8
Column: 250 × 4.6 10 μm Chiralcel OD-R (Optimize Technologies, USA)
Mobile phase: MeCN:300 mM aqueous sodium perchlorate 42:58
Flow rate: 0.5
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 20.2 (R), 22.9 (S)
Internal standard: diphenhydramine (13.8)
Limit of detection: 10 ng/mL
Limit of quantitation: 15 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; SPE; chiral

REFERENCE
Liu,J.; Stewart,J.T. Quantitation of trimipramine enantiomers in human serum by enantioselective high-performance liquid chromatography and mixed-mode disc solid-phase extraction, *J.Chromatogr.B*, **1997**, *700*, 175–182.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 800 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200 μL 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Guard column: μBondapak/Porasil
Column: μBondapak C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 13.68 g KH_2PO_4 in 2 L water, adjusted to pH 4.7 with dilute KOH.)
Column temperature: 50
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Internal standard: clomipramine
Limit of detection: 3 ng

KEY WORDS
plasma

REFERENCE
Wong,S.H.; Stolarun,S.L. Liquid-chromatographic analysis of trimipramine in plasma, *Clin.Chem.*, **1981**, *27*, 1101.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 200 μL 10 μg/mL protriptyline in water + 200 μL 80 g/L $NaHCO_3$ + 5 mL hexane, vortex for 15 s, centrifuge for 5 min. Remove the hexane layer and evaporate it in a stream of nitrogen at 60°. Reconstitute in 100 μL mobile phase, vortex for 15 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak CN
Mobile phase: MeCN:MeOH:5 mM phosphate buffer 60:15:25, adjusted to pH 7.0
Flow rate: 2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 2.33
Internal standard: protriptyline (12.20)
Limit of detection: 6 ng/mL

OTHER SUBSTANCES
Extracted: imipramine, doxepin, amitriptyline, desmethyldoxepin, nortriptyline, desipramine, chlorpromazine, procainamide, thioridazine, propranolol, propoxyphene, disopyramide, maprotiline
Noninterfering: caffeine, theophylline, salicylic acid, chlordiazepoxide, methaqualone, diazepam, acetaminophen
Interfering: trifluoperazine

KEY WORDS
serum

REFERENCE
Koteel,P.; Mullins,R.E.; Gadsden,R.H. Sample preparation and liquid-chromatographic analysis for tricyclic antidepressants in serum, *Clin.Chem.*, **1982**, *28*, 462–466.

SAMPLE
Matrix: blood
Sample preparation: Evaporate 200 μL 1 μg/mL clomipramine in MeOH into a tube, add 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100 μL 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:25 mM KH_2PO_4:water 45:50:5
Flow rate: 1
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM
Retention time: 10.10
Internal standard: clomipramine (13)
Limit of detection: 4-5 ng/mL

OTHER SUBSTANCES
Extracted: imipramine, desipramine
Noninterfering: norclobazam, triazolam, monodesmethyltrimipramine, flunitrazepam, alimemazine, alprazolam, amineptine, caffeine, carbamazepine, citalopram, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl loflazepate, indalpine, loprazolam, lorazepam, meprobamate, nitrazepam, nordiazepam, nortriptyline, oxazepam, viloxazine

Interfering: amitriptyline, clobazam, levomepromazine

KEY WORDS
plasma

REFERENCE
Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, *375*, 339–347.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elut C-18 SPE cartridge twice with MeOH and twice with water. 500 μL Serum + 50 μL 1 μg/mL N-propionylprocainamide in 2.5 mM HCl, add to SPE cartridge, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with 1 volume MeOH:2.5 mM HCl 10:90. Add 200 μL 10 mM acetic acid and 5 mM diethylamine in MeOH to column, let stand 1 min, elute under vacuum, repeat, evaporate eluents to dryness under nitrogen at room temperature, reconstitute in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: Pelliguard LC-CN (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-PCN
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28
Flow rate: 1.2
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 6.9
Internal standard: N-propionylprocainamide (6)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, desipramine, doxepin, imipramine, nortriptyline, protriptyline
Simultaneous: atropine, butalbital, chlorpromazine, maprotiline, methadone, norpropoxyphene, phenylpropanolamine, prochlorperazine, promethazine, propranolol, quinidine, trifluoperazine, trimeprazine
Noninterfering: acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil
Interfering: hydroxyamitriptyline, procainamide

KEY WORDS
serum; SPE

REFERENCE
Lin,W.-N.; Frade,P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1987**, *9*, 448–455.

SAMPLE
Matrix: blood
Sample preparation: Inject 200 μL serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

HPLC VARIABLES
Column: A 40 × 4 TSKprecolumn PW (Tosoh); B 150 × 4 TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate
Flow rate: 1
Injection volume: 200
Detector: UV 210

CHROMATOGRAM
Retention time: 19
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, amoxapine, clomipramine, desipramine, doxepin, imipramine, maprotiline, nortriptyline

KEY WORDS
serum; column-switching; use gradient to determine metabolites

REFERENCE
Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, *35*, 453–456.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Plasma + 20 ng chlorpromazine + 10 mL hexane:isoamyl alcohol 98:2, vortex, shake for 15 min, centrifuge at 840 g at 0-2° for 15 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb nitrile
Mobile phase: MeCN:MeOH:buffer 1:1:1 (Buffer was K_2HPO_4 adjusted to pH 6.5 with orthophosphoric acid.)
Column temperature: 40
Flow rate: 0.8
Detector: E, ESA Coulochem Model 5100A, electrode 1 +0.3 V. electrode 2 +0.85 V

CHROMATOGRAM
Retention time: 15
Internal standard: chlorpromazine (17)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Gulaid,A.A.; Jahn,G.A.; Maslen,C.; Dennis,M.J. Simultaneous determination of trimipramine and its major metabolites by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *566*, 228–233.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700 μL Serum + 50 μL 5 μg/mL protriptyline in 5% potassium bicarbonate + 700 μL MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL

MeCN, elute with 250 μL MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 15 mm 7 μm Brownlee RP-8
Column: 150 × 4.6 5 μm Ultrasphere Octyl
Mobile phase: MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid
Flow rate: 1.5
Injection volume: 50
Detector: UV 215

CHROMATOGRAM
Retention time: 9.6
Internal standard: protriptyline (6.6)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline, clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, maprotiline, nortriptyline

KEY WORDS
serum; SPE

REFERENCE
Gupta,R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 2751–2765.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum or plasma + 200 μL 0.33 M NaOH, shake 5 s, add 7 mL n-hexane:iso-amyl alcohol 985:15, shake 20 min, centrifuge at 2100 g for 5 min. Remove organic phase and add 200 μL 0.1 M HCl to it, shake for 1 min, discard organic phase, inject 30 μL of aqueous phase.

HPLC VARIABLES
Guard column: 10 mm 10 μm Bischoff C18
Column: 125 × 4 5 μm Ecotube Nucleosil C8
Mobile phase: MeCN:water:diethylamine:PicB5 370:630:0.4:25 (PicB5 is water-MeOH-1-pentanesulfonic acid.)
Column temperature: 55
Flow rate: 1.7
Injection volume: 30
Detector: UV 230

CHROMATOGRAM
Retention time: 8.4
Internal standard: trimipramine
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: fluoxetine, norfluoxetine
Noninterfering: alprazolam, bromazepam, clorazepate, diazepam, flunitrazepam, lorazepam, oxazepam, triazolam, amitriptyline, clomipramine, desipramine, imipramine, fluvoxamine, nortriptyline

KEY WORDS
serum; plasma; trimipramine is IS

REFERENCE

el Maanni,A.; Combourieu,I.; Bonini,M.; Creppy,E.E. Fluoxetine, an antidepressant, and norfluoxetine, its metabolite, determined by HPLC with a C_8 column and ultraviolet detection, *Clin.Chem.*, **1993**, *39*, 1749–1750.

SAMPLE

Matrix: blood

Sample preparation: Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300 μL 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300 μL 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100 μL aliquot of the aqueous phase.

HPLC VARIABLES

Guard column: LC-8-DB (Supelco)
Column: 150 × 4.6 LC-8-DB (Supelco)
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)
Flow rate: 2
Injection volume: 100
Detector: UV 228

CHROMATOGRAM

Retention time: 5.9
Internal standard: protriptyline (4)

OTHER SUBSTANCES

Extracted: chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, imipramine, lidocaine, maprotiline, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, nortriptyline, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, verapamil

Noninterfering: acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, bendroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phencyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocainide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

Interfering: acetazolamide, amitriptyline

KEY WORDS

plasma; SPE

REFERENCE

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

SAMPLE

Matrix: blood

Sample preparation: 990 μL Serum + 10 μL 14 μg/mL trimipramine in MeOH. Inject onto column A and elute with mobile phase A for 15 min then elute contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4.6 10 μm Hypersil MOS C8; B 250 × 4.6 5 μm Nucleosil 100 CN
Mobile phase: A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:5778:235 (sic, perhaps 188:577:235 ?)
Detector: UV 214

CHROMATOGRAM
Internal standard: trimipramine

KEY WORDS
serum; trimipramine is IS; column-switching

REFERENCE
Rao,M.L.; Staberock,U.; Baumann,P.; Hiemke,C.; Deister,A.; Cuendet,C.; Amey,M.; Härtter,S.; Kraemer,M. Monitoring tricyclic antidepressant concentrations in serum by fluorescence polarization immunoassay compared with gas chromatography and HPLC, *Clin.Chem.*, **1994**, *40*, 929–933.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma or serum at 3000 g for 5 min, inject a 100 μL aliquot on to column A and elute to waste withy mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 3 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 4 10 μm Hypersil CPS; B 20 × 4.6 5 μm Spherisorb CN + 250 × 4.6 5 μm Spherisorb CN
Mobile phase: A MeCN:water 5:95; B MeCN:MeOH:8 mM pH 6.2 phosphate buffer 58:19:23
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 15
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Noninterfering: biperiden, chlorprothixene, diazepam, flunitrazepam, fluvoxamine, haloperidol, lorazepam, maprotiline, moclobemide, paroxetine, perazine, risperidone

KEY WORDS
plasma; serum; column-switching

REFERENCE
Härtter,S.; Hermes,B.; Hiemke,C. Automated determination of trimipramine and N-desmethyltrimipramine in human plasma or serum by HPLC with on-line solid phase extraction, *J.Liq.Chromatogr.*, **1995**, *18*, 3495–3505.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 251

CHROMATOGRAM

Retention time: 9.78
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur--compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM

Retention time: 22.0
Internal standard: cianopramine (8.93)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Simultaneous: amitriptyline, amoxapine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine
Interfering: promethazine, benztropine

KEY WORDS

serum; whole blood; liver

REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL MeOH + 1 mL 200 mM pH 9.8 carbonate buffer + 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 150 μL mobile phase, inject a 75 μL aliquot. Urine. 1 mL Urine + 1 mL β-glucuronidase (7200 Fishman Units) in 20 mM pH 6.5 phosphate buffer, heat at 37° for 24 h, add 100 μL 10 M NaOH, add 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 1 mL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 C18 base-deactivated silica (BDS) (Keystone)
Column: 50 × 4.6 5 μm C18 base-deactivated silica (BDS) (Keystone)
Mobile phase: MeCN:water 90:10 containing 0.1% formic acid and 10 mM ammonium acetate
Flow rate: 1
Injection volume: 50-75
Detector: MS, PE Sciex API III, heated nebulized interface, corona discharge needle +4 μA, nebulizer probe 500°, nebulizing gas was air at 2 L/min and 80 psi, curtain gas flow was nitrogen at 0.9 L/min, sampling orifice +45 V, dwell time 400 ms, interface heater 60°, electron multiplier–3.7 kV, collision gas was argon 355 × 10exp12 atoms/cm^2, first quadrupole filter admits m/z 276 (cyclobenzaprine) and 295 (trimipramine, collisional fragmentation at second filter, monitor m/z 215 (cyclobenzaprine) and 208 (trimipramine) at third quadrupole filter

CHROMATOGRAM

Retention time: 2.2
Internal standard: trimipramine

OTHER SUBSTANCES

Extracted: cyclobenzaprine

KEY WORDS
plasma; trimipramine is IS

REFERENCE
Constanzer,M.; Chavez,C.; Matuszewski,B. Development and comparison of high-performance liquid chromatographic methods with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine, *J.Chromatogr.B*, **1995**, *666*, 117–126.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL MeOH + 1 mL 200 mM pH 9.8 carbonate buffer + 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL mobile phase, inject a 150 μL aliquot. Urine. 1 mL Urine + 1 mL β-glucuronidase (7200 Fishman Units) in 20 mM pH 6.5 phosphate buffer, heat at 37° for 24 h, add 100 μL 10 M NaOH, add 5 mL hexane, rotate for 15 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL mobile phase, inject a 150 μL aliquot.

HPLC VARIABLES
Guard column: 20 mm long C18 base-deactivated silica (BDS) (Keystone)
Column: 250 × 4.6 5 μm C18 base-deactivated silica (BDS) (Keystone)
Mobile phase: MeCN:buffer 50:50 (plasma) or 43:57 (urine) (Buffer was 0.085% phosphoric acid adjusted to pH 6.5 with triethylamine.)
Flow rate: 1
Injection volume: 150
Detector: UV 229

CHROMATOGRAM
Retention time: 10.5 (plasma), 12.8 (urine)
Internal standard: trimipramine

OTHER SUBSTANCES
Extracted: cyclobenzaprine

KEY WORDS
plasma; trimipramine is IS

REFERENCE
Constanzer,M.; Chavez,C.; Matuszewski,B. Development and comparison of high-performance liquid chromatographic methods with tandem mass spectrometric and ultraviolet absorbance detection for the determination of cyclobenzaprine in human plasma and urine, *J.Chromatogr.B*, **1995**, *666*, 117–126.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.943

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quin-

idine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 4 5 μm CHIRAL-AGP (ChromTech)
Mobile phase: Isopropanol:59 mM pH 4.0 acetate buffer 1:99
Flow rate: 0.9
Injection volume: 20
Detector: UV 225

CHROMATOGRAM

Retention time: k' 4.43, 7.32 (enantiomers)

KEY WORDS

chiral

REFERENCE

Hermansson,J.; Grahn,A. Optimization of the separation of enantiomers of basic drugs. Retention mechanisms and dynamic modification of the chiral bonding properties on an α_1-acid glycoprotein column, *J.Chromatogr.A*, **1995**, *694*, 57–69.

SAMPLE

Matrix: solutions
Sample preparation: Inject an aliquot of a 200 μM solution in MeOH.

HPLC VARIABLES

Column: 100 × 4.7 7 μm Hypercarb (Shandon)
Mobile phase: MeOH containing 5 mM N-benzyloxycarbonylglycyl-L-proline and 4.5 mM NaOH
Column temperature: 17
Injection volume: 20
Detector: UV 270

CHROMATOGRAM

Retention time: k' 21 (first enantiomer)

KEY WORDS

chiral; α = 1.19

REFERENCE

Huynh,N.-H.; Karlsson,A.; Pettersson,C. Enantiomeric separation of basic drugs using N-benzyloxycarbonylglyclyl-L-proline as counter ion in methanol, *J.Chromatogr.A*, **1995**, *705*, 275–287.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 15.49 (A), 7.66 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, meronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Make up a 500 ng/mL solution in mobile phase, inject an aliquot.

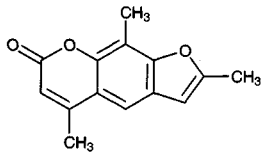
HPLC VARIABLES
Column: 250 × 4.6 5 μm Sumchiral OA-4700 (S)-tert-leucine-(R)-1-(α-naphthyl)ethylamine (YMC)
Mobile phase: Hexane:1,2-dichloroethane:EtOH:trifluoroacetic acid 1200:150:100:1
Flow rate: 1
Injection volume: 100
Detector: F ex 254 em 280 (filter)

CHROMATOGRAM
Retention time: 8.4, 9.3 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ponder,G.W.; Butram,S.L.; Adams,A.G.; Ramanathan,C.S.; Stewart,J.T. Resolution of promethazine, ethopropazine, trimeprazine and trimipramine enantiomers on selected chiral stationary phases using high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *692*, 173–182.

Trioxsalen



Molecular formula: $C_{14}H_{12}O_3$
Molecular weight: 228.25
CAS Registry No.: 3902-71-4
Merck Index: 9864
Lednicer No.: 1 334

SAMPLE
Matrix: aqueous humor, blood, tissue, vitreous humor
Sample preparation: Homogenize skin in 1 M pH 9.0 sodium borate buffer. 2 mL Whole blood, skin homogenate, aqueous humor, or vitreous humor + 5 mL 1 M pH 9.0 sodium borate buffer, mix, add 16 mL n-hexane:isopropanol 95:5, shake on a reciprocating shaker at 70-80 strokes/min for 30 min, centrifuge at 1020 g for 20 min. Remove 10 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL EtOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 Micropak MCH-10 reverse-phase
Mobile phase: Gradient. MeCN:water (not otherwise specified)
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 16.5
Internal standard: 8-methoxypsoralen (8)
Limit of detection: 2 ng/mL

KEY WORDS
whole blood; guinea pig; skin; human

REFERENCE
Chakrabarti,S.G.; Grimes,P.E.; Minus,H.R.; Kenney,J.A.,Jr.; Pradhan,T.K. Determination of trimethylpsoralen in blood, ophthalmic fluids, and skin, *J.Invest.Dermatol.*, **1982**, *79*, 374–377.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + diazepam, extract with heptane:dichloromethane 80:20.

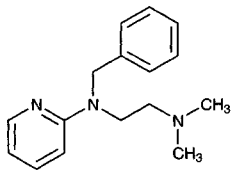
HPLC VARIABLES
Column: C18
Mobile phase: MeOH:water 70:30
Detector: UV 254 or F ex 360 em 430

CHROMATOGRAM
Limit of detection: 1 μg/mL (UV)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Stolk,L.; Siddiqui,A.H.; Cormane,R.H. Serum levels of trimethylpsoralen after oral administration, *Br.J.Dermatol.*, **1981**, *104*, 443–445.

Tripelennamine



Molecular formula: $C_{16}H_{21}N_3$
Molecular weight: 255.36
CAS Registry No.: 91-81-6, 6138-56-3 (citrate), 154-69-8 (HCl)
Merck Index: 9868
Lednicer No.: 1 51

SAMPLE
Matrix: blood, milk
Sample preparation: Centrifuge milk at 1200 g, remove the middle aqueous layer. 1 mL Plasma or milk + 50 μL MeOH:water 50:50 + 50 μL 4 μg/mL protriptyline in MeOH:water 50:50, mix, inject a 250 μL aliquot of this mixture on to column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 37-50 μm 10 × 1.5 Corasil RP C18; B 100 × 4 Techsphere 3CN (HPLC Technology)
Mobile phase: A water; B MeCN:50 mM pH 7.2 acetate buffer 70:30
Flow rate: A 0.8; B 0.9
Injection volume: 250
Detector: UV 246

CHROMATOGRAM
Retention time: 5.2
Internal standard: protriptyline (6.8)
Limit of detection: 2 ng/mL

KEY WORDS
column-switching; cow; plasma

REFERENCE
Dadgar,D.; Power,A. Applications of column-switching techniques in biopharmaceutical analysis. II. High-performance liquid chromatographic determination of tripelennamine in bovine plasma and milk, *J.Chromatogr.*, **1987**, *421*, 216–222.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254